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| FORM PTO-1390 (REV. 1-98) | | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER EMU144NAT |
| TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371 | | | U.S. APPLICATION NO. (If known, see 37 CFR 1.5) unknown |
| | | | 09/582375 |
| INTERNATIONAL APPLICATION NO. PCT/US98/14979 | INTERNATIONAL FILING DATE 20 July 1998 (20.07.98) | PRIORITY DATE CLAIMED 19 August 1997 (19.08.97) | |
| TITLE OF INVENTION NOSCAPINE DERIVATIVES, USEFUL AS ANTICANCER AGENTS | | | |
| APPLICANT(S) FOR DO/EO/US JOSHI, Harish C.; YE, Keqiang; KAPP, Judith; LIU, Fuqiang | | | |
| <p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <input type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 37 (b) and PCT Articles 22 and 39(1). <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input type="checkbox"/> A copy of the International Application as published (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unexecuted) <input type="checkbox"/> A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.197 and 1.98 <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> Other items or information: Certificate of Mailing under Express Mail Label No. EL209598034US Submission of Photocopies of Transmittal Documents and Request for Entry Into National Phase Under 35 U.S.C. 371 | | | |

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IN THE U.S. RECEIVING OFFICE
FOR THE PATENT COOPERATION TREATY

Applicants Harish C. Joshi, Keqiang Ye,
Judith Kapp, and Fuqiang Liu

International
Application No.: PCT/US98/14979

U.S. Serial No.: unknown

International
Filing Date: 20 July 1998 (20.07.98)

U.S. Filing Date: 18 February 2000

For: NOSCAPINE DERIVATIVES, USEFUL AS ANTICANCER
AGENTS

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Angela M. Rossi

Date: 08 June 2000

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STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(d)) -- NONPROFIT ORGANIZATION

Docket Number: EMU144NAT

Applicant, Patentee, or Identifier: Harish C. Joshi, et al.
Application or Patent No.: U.S. National Phase of PCT/US98/14979
Filed or Issued: February 18, 2000
Title: Noscapine Derivatives, Useful as Anticancer Agents

I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below:

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ADDRESS OF NONPROFIT ORGANIZATION 1380 So. Oxford Road
Atlanta, Georgia 30322

TYPE OF NONPROFIT ORGANIZATION:

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- ☐ the specification filed herewith with title as listed above.
☒ the application identified above.
☐ the patent identified above.

I hereby state that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities and that no rights to the invention are held by any person, other than the inventor, that would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OR PERSON SIGNING: Nancy L. Wilkinson, M.P.H.
TITLE IN ORGANIZATION OF PERSON SIGNING: Assistant Vice President for Research
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SIGNATURE:  DATE February 1, 2000

09/582375

NOSCAPINE DERIVATIVES, USEFUL AS ANTICANCER AGENTS

BACKGROUND OF THE INVENTION

5 The research conducted for the present invention was supported in part by a grant from National Institute of Health.

Two important events in the cell division cycle are the duplication of the chromosomal DNA and the separation of the duplicated chromosomes. These events occur in two discrete phases: the synthetic phase (S-phase) and the mitotic phase (M-phase), which are separated from each other by distinct gaps in time, gap 1 (G1) and gap 2 (G2). The proper coordination of these events is achieved by checkpoint pathways that delay the progression of the cell cycle when proper completion of one phase is disrupted by physical damage or other means. Under normal circumstances, if the extent of damage is irreparable, most cells initiate a sequence of biochemical events leading to programmed cell death or apoptosis. Deregulation in any one or more of these checkpoint mechanisms sometimes leads to genetic instability which is a primary step for a tumor to evolve into invasive malignant state. The chemotherapeutic management of various cancers is achieved by drugs that block either the S-phase, the M-phase, or that block regulatory or metabolic pathways impinging upon the cell cycle machinery. For example, some drugs affect the functions or structures of DNA or RNA, others interfere with enzymes involved in folate, purine, or pyrimidine metabolism, or the function of mitotic spindles. Anti-mitotic drugs such as vinca alkaloids and taxoids can arrest cells in M-phase by interacting with mitotic spindle components, microtubules. Microtubules are one of the major filamentous components of the cytoskeleton, and, together with actin and intermediate filaments, they organize the cellular cytoplasm. In interphase cells a dynamic radial array of microtubules emanates from the centrosome at the cell center. In this array, the fast growing and fast shrinking plus ends of microtubules project distally from the center. During mitosis, the duplicated centrosomes nucleate assembly of much more dynamic and more numerous polymers as they move apart to form the opposite poles of the mitotic spindle. The increased dynamics and number of microtubules enhance the chance-encounter of growing microtubules with the primary construction of the duplicated chromatid pairs. Upon attaching to microtubules, chromosomes undergo a series of

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movements eventually leading to their conversion and final assembly at the mid-plate during metaphase. The onset of the next event in mitosis, the anaphase, is delayed until each of the chromatid pairs is assembled at the metaphase mid-plate and proper tension is generated on the attached sister chromatids.

5 Dynamic assembly or disassembly of microtubules is required for the morphogenesis of mitotic spindle. Accordingly, small organic molecules that modulate the dynamics of microtubules primarily because some of the microtubule interacting agents are useful for chemotherapeutic management of certain kinds of tumors. There are two classes of these anti-microtubule agents: those that prevent the assembly of tubulin, and
10 those that promote the assembly of tubulin. A prototypic example of a potent assembly inhibitor is colchicine. Others are analogs of colchicine such as podophyllotoxin, MTC [(2-methoxy-5-(2,3,4-trimethoxyphenyl)-2,4,6-cycloheptatrien-1-one)], TCB (2,3,4-trimethoxy-4'-carbomethoxy-1,1'-biphenyl) and TKB (2,3,4-trimethoxy-4'-acetyl-1,1'-biphenyl), and vinca alkaloids. Taxol and its analogs represent a class of compounds that
15 promote the assembly of microtubules. It is now clear that although all of these microtubule drugs prevent cell division, only a select few have been useful clinically. In addition, there are differences regarding the toxicity and the efficacy of these drugs for distinct classes of tumors.

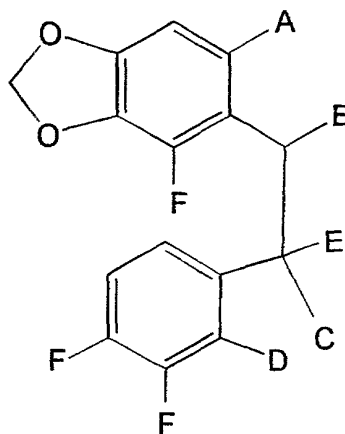
 Applicants have discovered that the antitussive noscapine and its
20 derivatives are useful in the treatment of neoplastic diseases. Noscapine is used as an antitussive drug and has low toxicity in humans. Noscapine arrests mammalian cells at mitosis, causes apoptosis in cycling cells, and has potent antitumor activity. Noscapine is an alkaloid from opium, and is readily available as a commercial byproduct in the commercial production of prescription opiates. Applicants have unexpectedly discovered
25 that noscapine promotes assembly of tubulin subunits.

BRIEF DESCRIPTION OF THE INVENTION

 Applicants have synthesized derivatives of noscapine, a known antitussive having low toxicity in humans, and have shown they promote assembly of tubulin subunits,
30 a characteristic suitable for the treatment of tumours and various neoplastic diseases.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a pharmaceutical composition comprising a
 5 compound of the formula



wherein: A is

10 (i) $(\text{CH}_2)_1\text{-N}-\text{C}(\text{O})\text{O}-\text{C}_{1-6}\text{ alkyl}$; and W is $\text{C}_{1-6}\text{ alkyl}$;

15 (ii) $(\text{CH}_2)_2\text{-N}-$ and forms a six membered ring

with B, said ring containing one nitrogen;

20 Y is

(a) $\text{C}_{1-6}\text{ alkyl}$, or H;

(b) $\text{C}(\text{O})-\text{C}_{1-6}\text{ alkyl}$;

(c) $\text{CH}_2\text{CH}(\text{OH})\text{-CH}_2\text{-Z}$, wherein Z is $\text{C}_{1-6}\text{ alkyl}$ or $\text{O}-\text{C}_{1-6}\text{ alkyl}$;

25 (d) aryl; or

(e) heterocycle;

B is a single bond, OH or halo;

5 C is -OH, -CH₂- or forms a 5-membered lactone or lactam ring with D; and

D is:

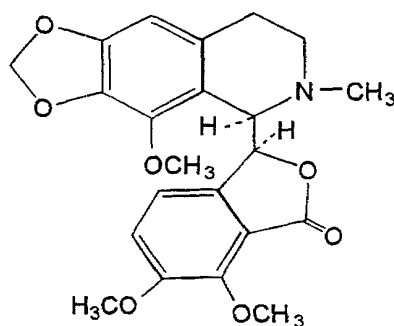
(i) -OH, -CH₂-halo, -CH(O)-, -COOH, -C(O)-O-C₁₋₆ alkyl, -(CH₂)_n,
-CHOH-, wherein n is an integer and is 1, 2, or 3; or

10 (ii) forms a 5-membered lactone or lactam ring with D;

E is -H or -CH₃; and

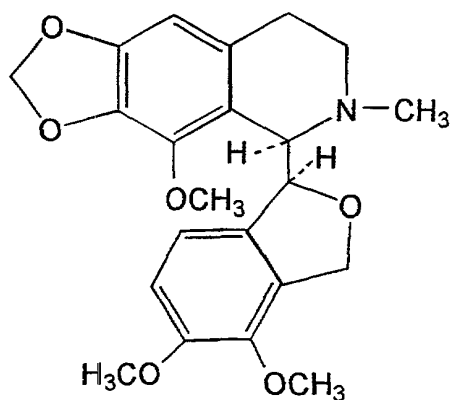
F is -OH or -OCH₃,

or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier,
15 said composition useful in the treatment of neoplastic diseases,
with the proviso that the formula excludes noscapine of the structure



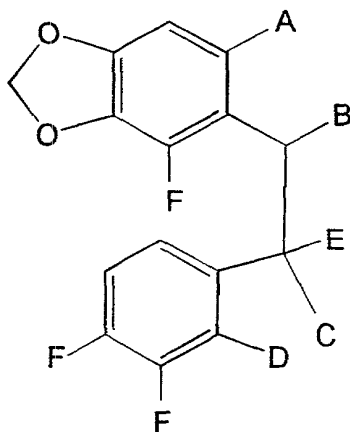
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25 In the present invention, one preferred compound is :

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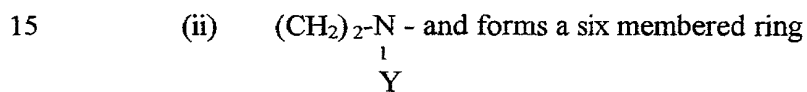
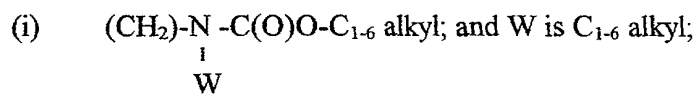


or pharmaceutically acceptable salt thereof.

The present invention also relates to a method for the treatment of
 5 neoplastic diseases, comprising administering to a mammal in need of such treatment an
 effective amount of a composition comprising a compound of the formula



10 wherein: A is



with B, said ring containing one nitrogen;

Y is

- (a) C_{1-6} alkyl, or H;
- (b) $C(O)-C_{1-6}$ alkyl;
- (c) CH_2CH-CH_2-Z , wherein Z is C_{1-6} alkyl or $O-C_{1-6}$ alkyl;
 $\quad \quad \quad |$
 $\quad \quad \quad OH$
- (d) aryl; or
- (e) heterocycle;

B is a single bond, OH or halo;

C is -OH, $-CH_2-$ or forms a 5-membered lactone or lactam ring with D; and

D is:

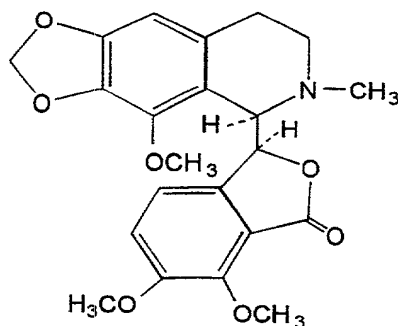
- (i) -OH, $-CH_2$ -halo, $-CH(O)-$, $-COOH$, $-C(O)-O-C_{1-6}$ alkyl, $-(CH_2)_n-$, $-CHOH-$, wherein n is an integer and is 1, 2, or 3; or
- (ii) forms a 5-membered lactone or lactam ring with D;

E is -H or $-CH_3$; and

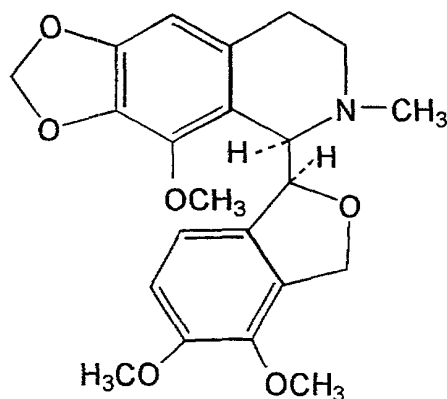
F is -OH or $-OCH_3$,

or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, said composition useful in the treatment of neoplastic diseases,

with the proviso that the formula excludes noscapine of the structure



In the method of the present invention, one preferred compound is



5 or pharmaceutically acceptable salts thereof.

The compounds of the present invention, may have asymmetric centers and occur as racemates, racemic mixtures and as individual diastereomers or enantiomers, with all isomeric forms being included in the present invention.

10 When any variable (e.g., W, Y, A, B, C, etc.) occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

15 As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms (Me is methyl, Et is ethyl, Pr is propyl, Bu is butyl); "Halo" as used herein means fluoro, chloro, bromo and iodo. As used herein, with exceptions as noted, "aryl" is intended to mean phenyl (Ph) or naphthyl.

20 The term heterocycle or heterocyclic, as used herein except where noted, represents a stable 5- to 7-membered mono- or bicyclic or stable 7- to 10-membered bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and

including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include but are not limited to piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodiny, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidinyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazoliny, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazoyl, benzopyranyl, benzothiazolyl, benzoxazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl.

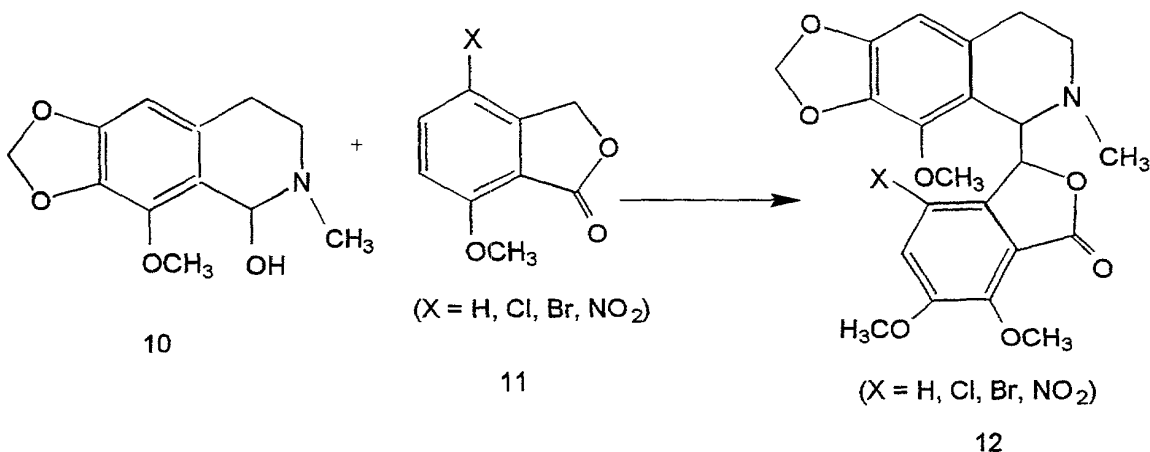
The pharmaceutically-acceptable salts of the compounds of the present invention (in the form of water- or oil-soluble or dispersible products) include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate, aspartate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methansulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others.

Synthesis of Noscapine

Noscapine is an alkaloid occurring in abundance in the opium plant, Papaver somniferum L. papaveraceae. It can be extracted from the water-insoluble residue remaining from the processing of opium in the commercial synthesis of morphine. It is readily available commercially in large quantities at low cost, from e.g., Aldrich Chemical Co., or Sigma Chemical Co. Noscapine can be separated from other opium alkaloids by the procedure according to Al-Yuhya, M.A. et al., in K. Florey (Ed.) *Analytical Profiles of Drug Substances*, Vol. 11 Academic Press 1982, pp. 407-461, or Sim, S.K. "Medicinal Plant Alkaloids," 2nd Ed. Un. Toronto Press 1970, p. 70.

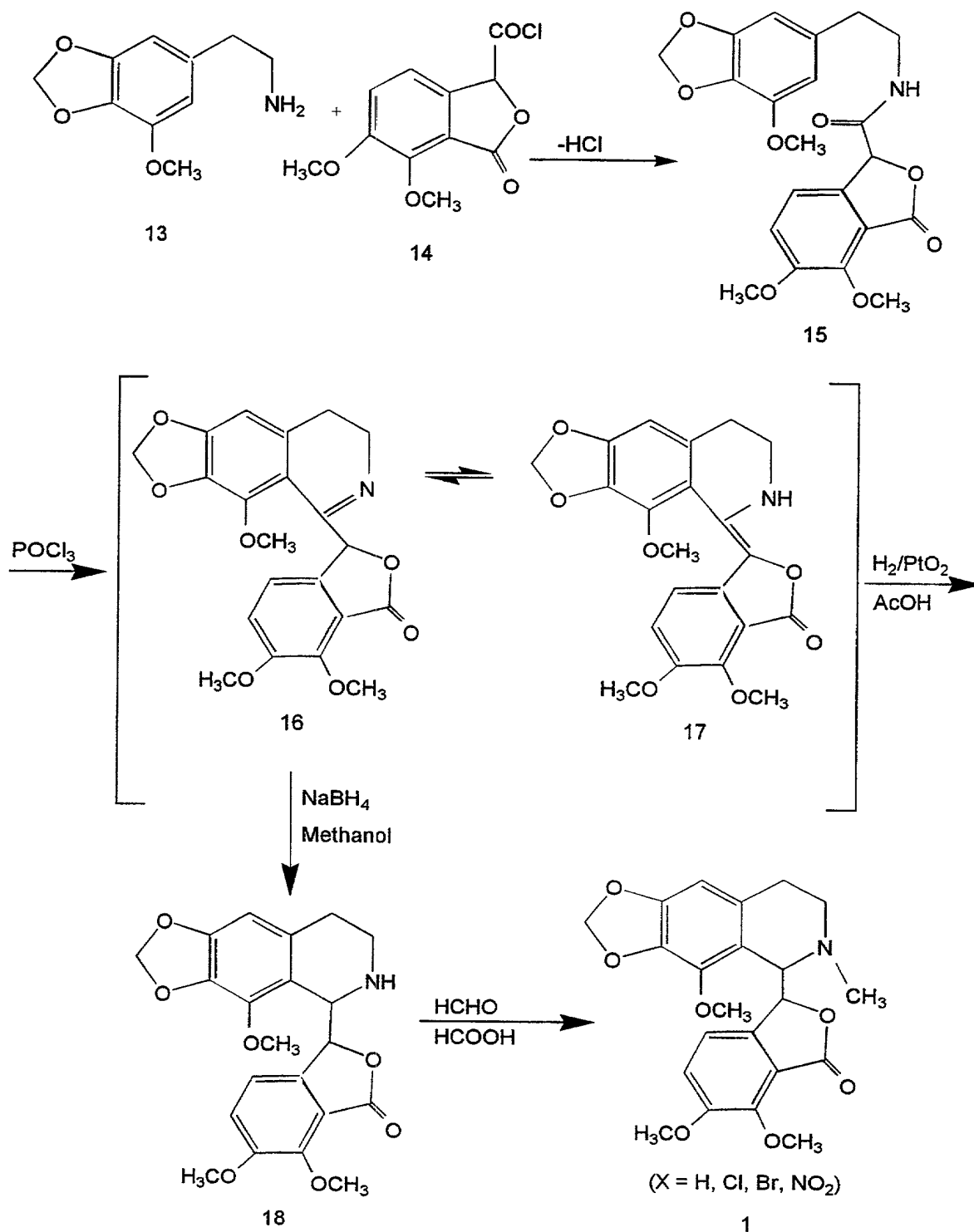
Chemical synthesis of noscapine **1** is less desirable, although feasible. See, for example, Fleischhacker, W. et al. *Chem. Monthly* 120, 765 (1989); Shono, T. et al. *Tetrahedron Lett.* 21, 1351 (1980).

There are a variety of methods to synthesize noscapine. A one step synthetic reaction was published by W. H. Perkin and R. Robinson, *J. Chem. Soc.* [London], **99**, 775 (1911). However, this method gave low yield and racemic mixtures. The reaction is shown as follows:



A second method was published by Von P. Kereks and R. Bognar. *J. Prakt. Chem.* **313**, 923-928 (1971). In this method, 2-(3'-methoxy-4',5'-methylenedioxy-phenyl) ethylamine **13** reacts with meconine-3-carbonyl chloride **14** in benzene to give N-(β -3-methoxy-4,5-methylenedioxyphenylethyl)-mekonine-3-carboxamide **15** with a yield of 86.6%. Compound **15** was cyclized by boiling with POCl₃ for 5 hr to produce compounds **16** and **17** with a yield of 46.7%. Compounds **16** and **17** are two isomers from cyclization of compound **15**. These two isomers are reduced by either H₂/PtO₂ in acetic acid, or NaBH₄ in methanol. The reduced compound **18** was methylated by boiling with the mixture of HCHO and HCOOH, to produce noscapine **1** with a yield of 20.3%.

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The compounds of the present invention are useful in the treatment of tumor cells and a variety of cancers, including but not limited to cancer of the colon, non-small cell lung cancer, cancer of the brain, ovarian cancer, cancer of the kidney, cancer of the prostate, leukemia, breast cancer, cancer of the bladder. For most of these kinds of neoplastic diseases, applicants have tested a variety of cell lines with noscapine, or derivatives thereof.

For these purposes, the compounds of the present invention may be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques), by inhalation spray, or rectally, in dosage unit formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Thus, in accordance with the present invention there is further provided a method of treating and a pharmaceutical composition for treating tumor cells and related cancers. The treatment involves administering to a patient in need of such treatment a pharmaceutical composition comprising a pharmaceutical carrier and a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

These pharmaceutical compositions may be in the form of orally-administrable suspensions or tablets; nasal sprays; sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions or suppositories.

When administered orally as a suspension, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

When administered by nasal aerosol or inhalation, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and

may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

Dosage levels of the order of 0.02 to 5.0 or 10.0 grams-per-day are useful in the treatment or prevention of the above-indicated conditions, with oral doses two-to-five times higher. For example, compound 4 is effectively treated by the administration of from 10 to 50 milligrams of the compound per kilogram of body weight from one to three times per day. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

EXAMPLE 1

Synthesis of Noscapine

Noscapine 1 was synthesized by the methods of Shono, T. et al., Tetrahedron Lett. 21, 1351 (1980); Fleishchacker, W. et al., Monatshefte fur Chemie 120, 765 (1989); Sam, J. et al., J. Pharm. Sci. 57: 1755 (1968); Al-Yuhya, M.A. et al., in K. Florey (Ed.) Analytical Profiles of Drug Substances, Vol. 11 Academic Press 1982, pp. 407-461; Battersby, A.R. et al., Tetrahedron Lett. 11, 669 (1965). It is readily available in

large quantities from a variety of commercial sources, e.g. Aldrich Chemical Co. or Sigma Chemical Co.

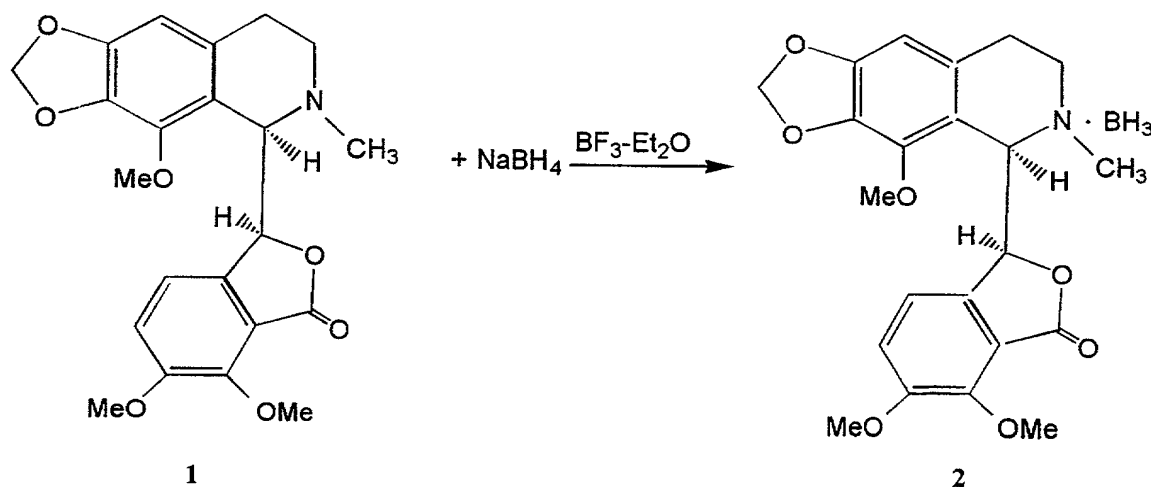
NMR data for (*S, R*)-Noscapine:

¹H NMR (CDCl₃, 300 MHz): δ 6.95 (d, *J*=8.1 Hz, 1 H), 6.27 (s, 1 H), 6.07 (d, *J*=8.4 Hz, 1 H), 5.90 (s, 2 H), 5.55 (d, *J*=3.9 Hz, 1 H), 4.37 (d, *J*=4.2 Hz, 1H), 4.06 (s, 3 H), 4.00 (s, 3 H), 3.83 (s, 3 H), 2.60 (m, 1 H), 2.52 (s, 3 H), 2.38-2.27 (m, 2 H), 1.94-1.87 (m, 1 H).

¹³C NMR (CDCl₃, 75.5 MHz): δ 168.0, 152.1, 148.3, 147.5, 140.9, 140.3, 133.9, 131.9, 120, 118.0, 117.6, 116.9, 102.2, 100.7, 81.7, 62.1, 60.7, 59.3, 56.7, 49.9, 46.2, 27.9.

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EXAMPLE 2



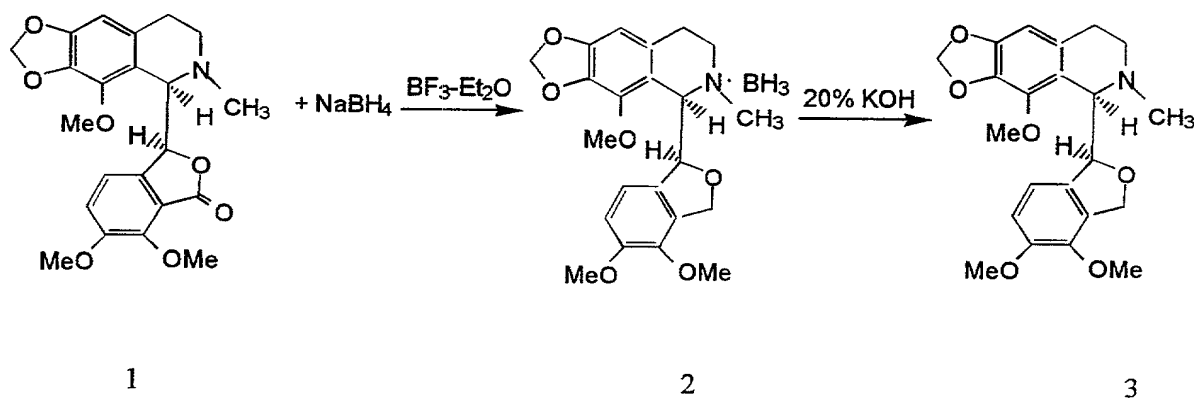
The synthesis of borane-(*S, R*)-noscapine complex (2):

(*S, R*)-Noscapine **1** (830mg, 2.0 mmol, 1.0 equiv.) was dissolved in 10 mL of BF₃-Et₂O. This solution was dropped slowly at 0° to a solution of NaBH₄ (150 mg, 4.0 mmol, 2.0 equiv.) in 14 mL dry THF and stirred at 0° for 1 h under N₂. Then it was refluxed for 2 h. After cooling to room temp, the solution was poured into ice water and extracted with CHCl₃ (70 mL x 2). The organic phase was washed with brine, dried with MgSO₄ and concentrated. The resulting oil was purified by flash chromatography (SiO₂, 3 x 15 cm, 50% EtOAc in hexane) to give **2** as a white solid (444 mg, 52%). TLC (silica

gel, 65% EtOAc in hexane, $R_f=0.75$); ^1H NMR (CDCl_3 , 300 MHz): δ 7.41 (d, $J=8.1$ Hz, 1 H), 7.31 (d, $J=8.1$ Hz, 1 H), 6.83 (s, 1 H), 6.33 (s, 1 H), 5.81 (dd, $J=12.9$ Hz, 0.9 Hz, 2 H), 4.54 (s, 1 H), 3.99 (s, 3 H), 3.92 (s, 3 H), 3.73 (m, 1 H), 3.20 (s, 3 H), 3.15 (m, 2 H), 2.93 (m, 1 H), 2.61 (s, 3H).

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EXAMPLE 3



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3

Compound 3 and 4 were prepared by literature method Prior, S.; Wiegreb, W. *Arch. Pharm.* **1983**, 316, 737.:

The synthesis of 1,3-dihydro-4,5-dimethoxy-1-[1-(8-methoxy-2-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolinyl)] isobenzofuran-BH₃ (3):

15

(*S,R*)-Noscaphine (830 mg, 2.0 mmol, 1.0 equiv.) was dissolved in 10 mL of $\text{BF}_3\text{-Et}_2\text{O}$. This solution was dropped slowly at 0°C to a solution of NaBH_4 (150 mg, 4.0 mmol, 2.0 equiv.) in 18 mL dry THF and stirred at 0°C for 1 h under N_2 . Then it was refluxed for 4 h. After cooling to room temp, the solution was poured into ice water and extracted with CHCl_3 (70 mL x 2). The organic phase was washed with brine, dried with MgSO_4 and concentrated. The resulting oil was purified by flash chromatography (SiO_2 , 3 x 15 cm, 50% EtOAc in hexane) to give 3 as a white solid (686 mg, 83%). TLC (silica gel, 50% EtOAc in hexane, $R_f=0.80$); IR (CH_2Cl_2 , NaCl , cm^{-1}) 2371 (s), 1616 (w). ^1H NMR (CDCl_3 , 300 MHz): δ 7.15 (d, $J=8.4$ Hz, 1 H), 6.93 (d, $J=8.1$ Hz, 1 H), 6.57 (br s, 1 H), 6.33 (s, 1 H), 5.79 (AB, $J=1.5$ Hz, 1 H), 5.74 (AB, $J=1.5$ Hz, 1 H), 4.81 (d, $J=12.0$

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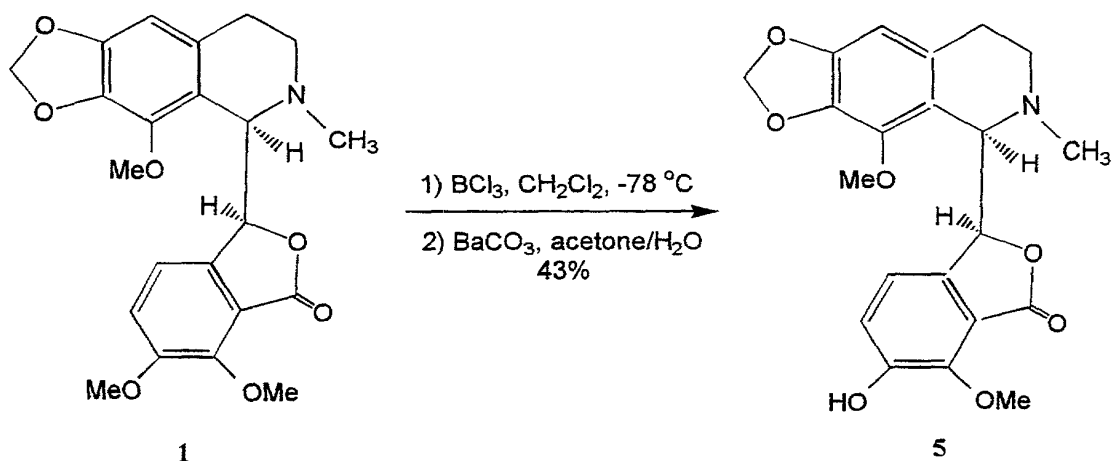
25

Hz, 1 H), 4.34 (s, 1 H), 4.07 (dd, $J=12.3$ Hz, 2.7 Hz, 1 H), 3.85 (s, 3 H), 3.71 (s, 3 H), 3.16 (s, 3 H), 3.05-2.81 (m, 4 H), 2.53 (s, 3 H). HRMS (FAB) Calcd for $C_{22}H_{28}BLiNO_6$ ($M+Li$)⁺: 420.2170, Found 420.2173.

- The synthesis of 1,3-dihydro-4,5-dimethoxy-1-[1-(8-methoxy-2-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolinyl)]isobenzofuran (**4**):
- Compound **3** (450 mg, 1.10 mmol) was refluxed in 15 mL of 20% aqueous KOH solution for 2 h. The reaction mixture was cooled to room temp, neutralized with 2 N HCl to PH=7 and extracted with $CHCl_3$. The organic phase was washed with brine, dried with $MgSO_4$ and concentrated. Compound **4** was crystallized from Et_2O (220 mg, 50%). ¹H NMR ($CDCl_3$, 300 MHz): delta 6.72 (d, $J=8.4$ Hz, 1 H), 6.32 (s, 2 H), 5.88 (m, 2 H), 5.63 (br s, 1 H), 5.02 (br s, 2 H), 4.40 (br s, 1H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.75 (s, 3 H), 3.14 (br s, 1 H), 2.64 (br s, 3 H), 2.60-2.43 (m, 3H).

EXAMPLE 4

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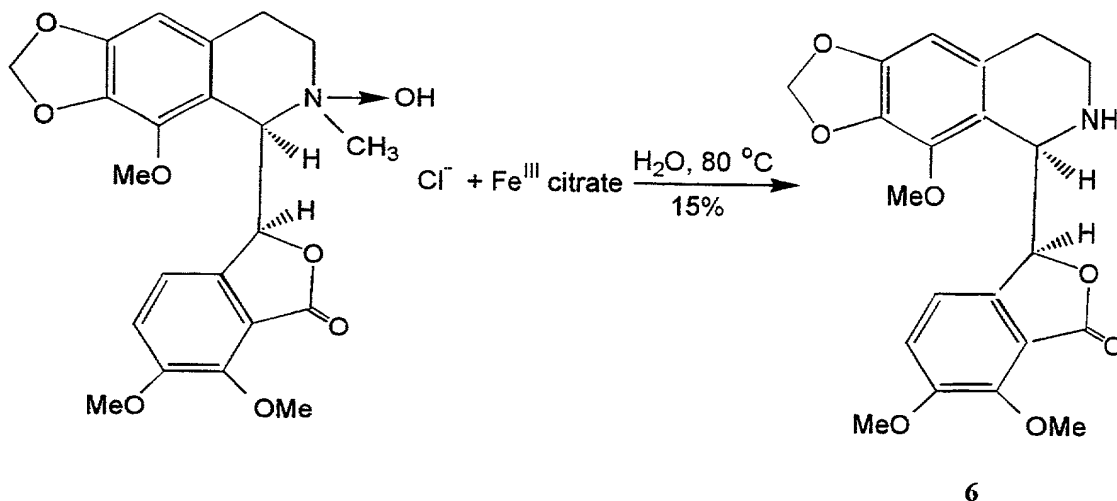
The synthesis of compound **5**:

- (*S,R*)-Noscipine (826 mg, 2.0 mmol, 1.0 equiv.) was dissolved in 25 mL of CH_2Cl_2 . This solution was added dropwise to a 1.0 M solution of borane trichloride in CH_2Cl_2 (8.0 mL, 8.0 mmol, 4.0 equiv.) at $-78^\circ C$. After 5 h, the reaction was quenched

with saturated aqueous NaHCO_3 (10 mL) and warmed to room temp. The reaction mixture was extracted with EtOAc (50 mL x 3), the combined organic phases were washed with brine (40 mL), dried with MgSO_4 and concentrated.

The obtained white solid was dissolved in acetone (50 mL) and H_2O (25 mL). This solution was treated with barium carbonate (1.55 g, 7.84 mmol) and refluxed for 3 h. After cooling to room temp, the reaction mixture was filtered. The filtrate was treated with 1 N HCl until $\text{pH}=2$, then extracted with EtOAc (50 mL x 3). The combined extracts were washed with brine, dried over MgSO_4 and concentrated. Crystallization ($\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$) was performed for the resulting off-white solid to give **5** as a silver gray solid (340 mg, 43%). IR (CH_2Cl_2 , NaCl, cm^{-1}) 3431 (s), 1767 (s). ^1H NMR (CDCl_3 , 300 MHz): δ 7.57 (br s, 1 H), 7.27 (br s, 1H), 6.57 (br s, 1 H), 6.33 (s, 1 H), 5.82 (s, 1 H), 5.78 (s, 1H), 5.11 (br s, 1 H), 4.08 (br s, 1 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.33 (m, 1 H), 3.20 (s, 3 H), 3.00 (m, 1 H), 2.82 (m, 2 H). ^{13}C NMR (CDCl_3 , 75.5 MHz): δ 166.3, 152.4, 150.1, 147.3, 139.8, 138.9, 133.4, 126.2, 119.3, 118.8, 116.9, 106.7, 102.3, 100.9, 78.5, 61.9, 58.2, 56.8, 45.2, 39.9, 21.4.

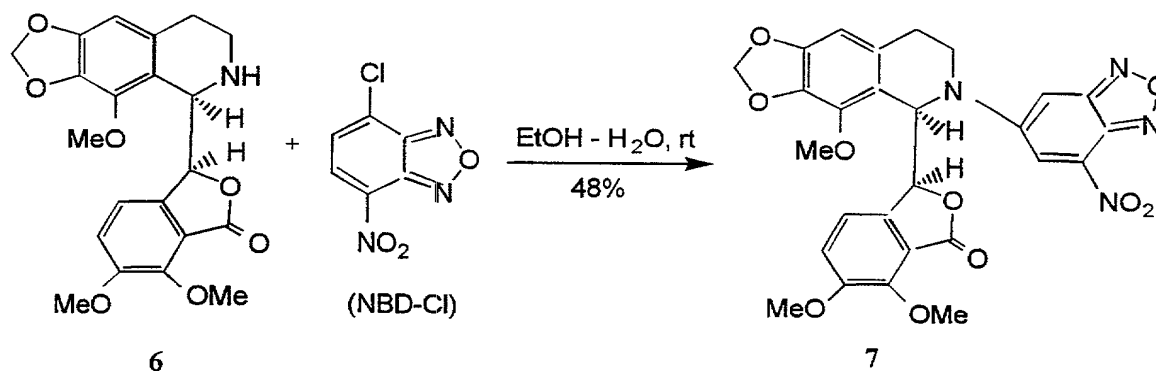
EXAMPLE 5



Ferric citrate (10.5 g) was dissolved in 200 mL of H_2O . Citric acid was added to make $\text{pH} = 2$. This solution was added to noscapine N-oxide HCl salt (6.0 g) [Uhrin, D. et al.,

Collect. Czech.Chem. Commun. 54:498(1989)] and the mixture was heated to 85°C for 3 h. After cooling to room temp, the solution was treated with saturated Na₂CO₃ solution until pH = 9 and extracted with CHCl₃ (200 mL x 4). The combined organic phases were washed with brine, dried with anhydrous MgSO₄ and concentrated. The resulting oil was purified by flash chromatography (SiO₂, 3 x 25 cm, 75% EtOAc in hexane) to give **6** as a yellow oil (793 mg, 15%). TLC (silica gel, 75% EtOAc in hexane, R_f=0.25); IR (CH₂Cl₂, NaCl, cm⁻¹) 3370 (w), 1758 (s); ¹H NMR (CDCl₃, 300 MHz): δ 6.92 (d, J = 8.1 Hz, 1 H), 6.29 (s, 1 H), 5.93-5.91 (m, 3 H), 5.87 (d, J = 3.9 Hz, 1 H), 4.80 (d, J = 3.9 Hz, 1 H), 4.04 (s, 3 H), 4.02 (s, 3 H), 3.80 (s, 3 H), 2.59-2.55 (m, 1 H), 2.50-2.38 (m, 1 H), 2.30-2.22 (m, 1 H), 2.15-2.07 (m, 1 H), 1.96 (br s, 1 H). ¹³C NMR (CDCl₃, 75.5 MHz): δ 168.4, 152.1, 148.3, 147.8, 141.0, 140.3, 134.1, 131.9, 119.5, 118.4, 117.5, 116.9, 103.1, 100.7, 80.6, 62.2, 59.4, 56.6, 52.7, 39.5, 29.6. HRMS (FAB) Calcd for C₂₁H₂₁LiNO₇ (M + Li)⁺: 406.1478, Found 406.1477. Anal. Calcd. for C₂₁H₂₁N₃O₇: C, 63.15; H, 5.30; N, 3.51. Found: C, 63.35; H, 5.45; N, 3.42.

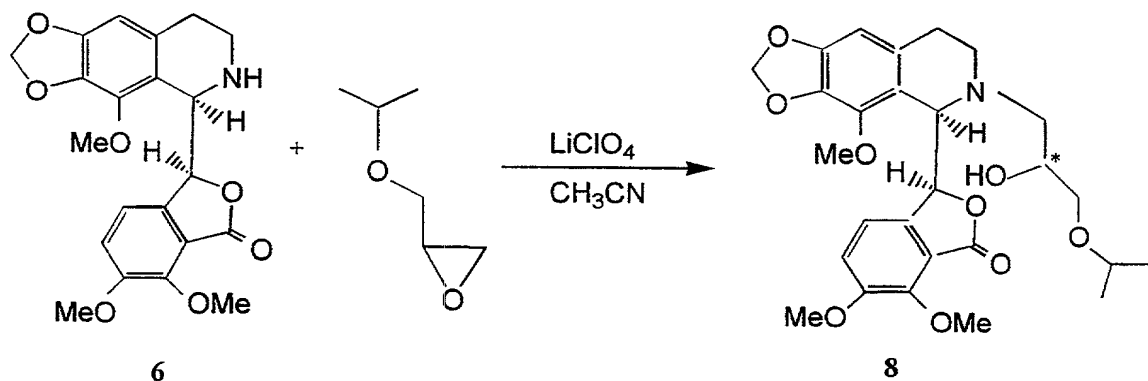
EXAMPLE 6



To an ETOH (15 mL) solution of amine **6** (160 mg, 0.40 mmol, 1.0 equiv.) was added 30 mL of Na₂B₄O₇ (1.14 g) buffer and NBD-CL (80 mg, 0.4 mmol, 1.0 equiv.). The reaction mixture was stirred at room temp for 15 h. Evaporation of ETOH left a dark orange slurry which was extracted with CHCl₃ (70 mL x 2). The combined organic phases were washed with brine, dried with MgSO₄ and concentrated. The resulting green oil was

purified by flash chromatography (SiO₂, 3 x 15 cm, 65 % EtOAc in hexane) to give an orange solid which was recrystallized from CH₂Cl₂ and hexane to give 7 as an orange crystal (108 mg, 48%). TLC (silica gel, 65% EtOAc in hexane, R_f=0.65); mp= 194-195° C (CH₂Cl₂/hexane); IR (CH₂Cl₂, NaCl, cm⁻¹) 1765 (s), 1616 (m), 1540 (s), 1500 (s), 1287 (s), 1261 (m). ¹H NMR (CDCl₃, 300 MHz): δ 8.53 (d, J = 9.0 Hz, 1 H), 7.41 (br d, J = 7.8 Hz, 1 H), 7.29 (d, J = 8.4 Hz, 1 H), 7.00 (br s, 1 H), 6.48 (d, J = 2.7 Hz, 1 H), 6.44 (s, 1 H), 6.04 (d, J = 2.7 Hz, 1 H), 5.90 (d, J = 7.5 Hz, 2 H), 4.02 (s, 3 H), 3.95 (s, 3 H), 3.81 (m, 1 H), 3.76 (s, 3 H), 3.70-3.50 (m, 2 H), 3.02-2.93 (m, 1 H). ¹³C NMR (CDCl₃, 75.5 MHz): δ 166.7, 152.8, 149.6, 147.9, 145.1, 145.0, 144.7, 139.5, 138.7, 135.3, 133.7, 130.4, 123.7, 119.0, 118.7, 118.1, 113.4, 102.9, 102.4, 100.9, 81.9, 62.2, 59.1, 58.1, 56.8, 46.5, 27.8. HRMS (FAB) Calcd for C₂₇H₂₂LiN₄O₁₀ (M + Li)⁺: 569.1496, Found 569.1472. Anal. Calcd. for C₂₇H₂₂N₄O₁₀: C, 57.65; H, 3.91; N, 9.96. Found: C, 57.85; H, 4.04; N, 9.81.

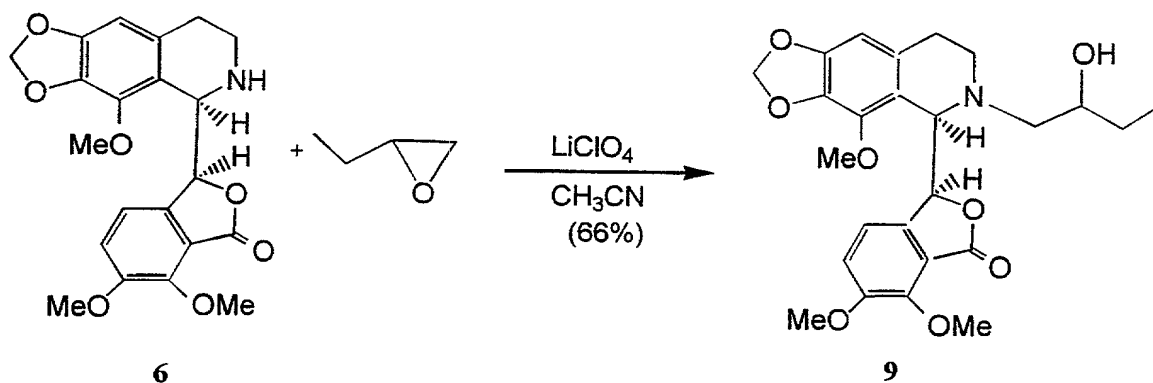
EXAMPLE 7



A CH₃CN (2 mL) solution of glycidyl isopropyl ether (50 μL, 0.40 mmol, 1.0 equiv.) was treated with anhydrous LiClO₄ salt (43 mg, 0.40 mmol, 1.0 equiv.) and stirred for about 10 min. until a clear solution was observed. This solution was treated with an CH₃CN (3 mL) solution of compound 6 (160 mg, 0.40 mmol, 1.0 equiv.) at room

temp. The mixture was refluxed for 24 h, cooled to room temp., washed with H₂O and extracted with Et₂O (40 mL x 3). The combined organic phases were washed with brine, dried with MgSO₄ and concentrated. The resulting orange oil was purified by flash chromatography (SiO₂, 2 x 15 cm, 50% EtOAc in hexane) to give **8** as a yellow oil which is a mixture of the two diastereomers (1:1 ratio, 172 mg, 83%). TLC (silica gel, 50% EtOAc in hexane, R_f=0.50); IR (CH₂Cl₂, NaCl, cm⁻¹) 3450 (m), 1761 (s), 1498 (m), 1478(m). ¹H NMR (CDCl₃, 300 MHz): δ 6.96 (d, J = 8.4 Hz, 2 H), 6.30 (s, 2 H), 6.24 (t, J = 8.4 Hz, 2 H), 5.90 (s, 4 H), 5.73 (d, J = 4.5 Hz, 1 H), 5.67 (d, J = 4.2 Hz, 1 H), 4.47 (d, J = 4.2 Hz, 1 H), 4.42 (d, J = 4.5 Hz, 1 H), 4.06 (s, 3 H), 4.05 (s, 3 H), 3.97 (s, 3 H), 3.96 (s, 3H), 3.82 (s, 6 H), 3.56 (d of sept, J = 6.0 Hz, 2 H), 3.41 (m, 4 H), 3.0 (br s, 2 H), 2.74-2.00 (m, 14 H), 1.12 (t, J = 5.7 Hz, 12 H). ¹³C NMR (CDCl₃, 75.5 MHz): δ 167.9, 167.8, 152.2 (2 C), 148.5 (2 C), 148.1 (2 C), 141.2, 141.0, 140.5, 140.4, 133.8, 133.7, 130.9, 130.5, 119.3, 119.0, 118.4 (2 C), 117.5 (2 C), 116.3, 115.8, 102.7, 102.6, 100.7, 100.6, 80.8, 80.1, 71.9, 71.8, 70.4, 70.1, 68.0, 67.8, 62.3, 62.2, 60.5 (2 C), 59.4, 59.2, 58.6, 58.5, 56.7 (2 C), 46.2, 44.7, 24.8, 24.3, 22.0 (2 C), 21.9 (2 C). Anal. Calcd. for C₂₇H₃₃NO₉: C, 62.90; H, 6.45; N, 2.72. Found: C, 62.97; H, 6.45; N, 2.64.

EXAMPLE 8



An CH₃CN (2 mL) solution of 1,2-epoxybutane (114 μL, 1.32 mmol, 3.3 equiv) in a sealed tube was treated with anhydrous LiClO₄ salt (43 mg, 0.40 mmol, 1.0

equiv) and stirred for 10 min until a clear solution was observed. This solution was treated with an CH_3CN (3 mL) solution of compound 6 (160 mg, 0.40 mmol, 1.0 equiv) at room temp. The mixture was heated at 115°C for 10 h, cooled to room temp, washed with H_2O and extracted with Et_2O (50 mL x 3). The combined organic phases were washed with
5 brine, dried with MgSO_4 and concentrated. The resulting yellow oil was purified by flash chromatography (SiO_2 , 2 x 15 cm, 65% EtOAc in hexane) to give 9 as a light yellow oil which is a mixture of the two diastereomers (1:1 ratio, 125 mg, 66%). TLC (silica gel, 65% EtOAc in hexane, $R_f = 0.60$); IR (CH_2Cl_2 , NaCl, cm^{-1}) 3523 (s), 1758(s), 1622 (m). ¹H NMR (CDCl_3 , 300 MHz): δ 6.99 (d, $J = 8.1$ Hz, 2 H), 6.31 (s, 1 H), 6.30 (s, 1 H),
10 6.26 (d, $J = 8.4$ Hz, 1 H), 6.25 (d, $J = 8.4$ Hz, 1 H), 5.90 (s, 4 H), 5.79 (d, $J = 4.5$ Hz, 1 H), 5.68 (d, $J = 3.9$ Hz, 1 H), 4.48 (d, $J = 3.9$ Hz, 1 H), 4.40 (d, $J = 4.5$ Hz, 1 H), 4.07 (s, 3 H), 4.06 (s, 3 H), 3.98 (s, 6 H), 3.83 (s, 6 H), 3.66-3.53 (m, 2 H), 3.20 (br s, 2 H), 2.68-2.38 (m, 8 H), 2.23-2.10 (m, 4 H), 1.42 (m, 4 H), 0.95 (t, $J = 6.9$ Hz, 3 H), 0.93 (t, $J = 7.2$ Hz, 3 H). ¹³C NMR (CDCl_3 , 75.5 MHz): δ 168.0, 167.7, 152.3, 152.2, 148.6 (2 C),
15 148.2, 148.1, 141.2, 140.9, 140.6, 140.3, 133.9, 133.7, 130.9, 130.1, 119.3, 118.8, 118.5 (2 C), 117.4 (2 C), 116.4, 115.4, 102.8, 102.5, 100.7, 100.6, 81.1, 79.6, 68.9, 68.7, 62.5, 62.3, 62.2, 61.3, 61.2, 59.3, 59.2, 57.5, 56.7 (2 C), 46.4, 44.0, 27.5, 27.1, 25.1, 23.7, 10.0, 9.8. Anal. Calcd. for $\text{C}_{25}\text{H}_{29}\text{NO}_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.68; H, 6.18; N, 2.90.

EXAMPLE 9

Noscapine Arrests Hela and Thymocyte cells at M Phase

Hela cells were grown in DMEM supplemented with 10% fetal calf serum, 1 mM L-glutamine and 1% penicillin/streptomycin. The tumor cell line E.G7-OVA (H-2^b)
25 [Moore, M.W., et al., *Cell* 54,777 (1988)] was grown in RPMI 1640 with 10% fetal calf serum, 1% sodium pyruvate, 1 mM L-glutamine, 0.1% gentamycin, 50 μM β -mercaptoethanol. Cells were grown at 37°C in a 5% CO_2 atmosphere. Cell viability was assessed by trypan blue exclusion analysis. Cell numbers were determined using a hemacytometer. C57BL/6 (H-2^b) mice, 8 to 12 weeks of age, were obtained from Harlan
30 Sprague Dawley, Inc. (Indianapolis, IN). Mice were maintained on standard laboratory

chow and water ad libitum in a temperature and light controlled environment. For immunofluorescence, both Hela and thymocyte cells were treated identically except the Hela cells were grown onto glass coverslips while EL4 thymocytes were put on glass coverslips after fixation. Cells in 10 ml medium were incubated with 2 μ l DMSO or 20 μ M Noscapine (2 μ l 0.1 M DMSO solution) respectively. After 24 hr, cells were fixed with cold (-20°C) methanol for 5 min., then rehydrated by PBS for 1 min. Nonspecific sites were blocked by incubating with 200 μ l of 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) at 37°C for 15 minutes. A mouse monoclonal antibody against α -tubulin was diluted 1:200 in PBS containing 1% BSA and incubated (200 μ l) with the coverslips at 37°C for 1 hr. Then cells were washed with 1% BSA-PBS solution for 10 min. at room temperature before incubating with a 1:200 dilution of a rhodamine labeled goat anti-mouse IgG antibody at room temperature for 45 min., then the coverslips were rinsed by 1% BSA/PBS solution for 10 min. and labeled by DAPI (4', 6-diamino-2-phenylindole) for another 10 min. at room temperature. The coverslips containing the cells were then mounted with a solution containing 0.01% 1,4-diazobicyclo (2,2,2) octane. Cells were examined with a fluorescence microscope. The flow cytometric evaluation of the cell cycle status was performed according to a modification of as described in Empey, D.W., et al., Eur. J. Clin. Pharmacol. 16, 393 (1979). Briefly, untreated or noscapine-treated cells were centrifuged, washed twice with ice-cold PBS, and fixed in 70% ethanol. Tubes containing the cell pellets were stored at -20°C for at least 24 hr. Following this, the cells were centrifuged at 1000 x g for 10 min. and supernatant was discarded. The pellets were resuspended in 30 μ l phosphate-citrate buffer at room temperature for 30 min. Cells were then washed with 5 ml PBS and incubated with propidium iodide and RNase (20mg/ml PI and 20 mg/ml RNase A in PBS) for 30 min. The samples were read on a cytometer.

Results show that by immunofluorescence, with an antibody specific for alpha-tubulin, that after treatment with noscapine, microtubule arrays are arrested in M phase in Hela and thermocyte cells. Flow cytometric analysis of DNA content showed consistent results.

EXAMPLE 10Noscapine Initiates Apoptosis

Oligonucleosomal fragmentation of genomic DNA was determined according to Walton, M.I. et. al., Cancer. Res. 53, 1853 (1993). An aliquot of 3.3×10^6 cells in 10 ml medium was incubated with 20 μ M Noscapine (2 μ l 0.1 M DMSO solution) for different time periods ranging from 0 to 24 hr. At the end of incubation, cells were pelleted and washed twice with ice-cold PBS, and lysed in 250 μ l 1% (v/v) NP-40 detergent containing 0.5 mg/ml proteinase K in PBS solution on ice for 60 min. Samples were centrifuged, and the supernatants were removed and incubated with 5 μ l 10 mg/ml Rnase A at 37°C for 40 min. An aliquot of 1 ml anhydrous ethanol was added, tubes were placed at -20°C for 20 min., then centrifuged to pellet DNA. After the samples were dry, the same amount of DNA (10 μ g) was electrophoresed at 80V for 3 hr. through a 2% agarose gel containing ethidium bromide in TAE buffer. DNA bands were visualized under HV light. A 123 bp DNA ladder was used as molecular size marker.

Morphological changes in the nuclear chromatin of cells undergoing apoptosis were detected by staining with 4', 6-diamidino-2-phenylindole (DAPI). In brief, 0.5×10^6 to 3×10^6 cells were fixed with 4% glutaraldehyde, 0.2% Triton x-100, in PBS and incubated at room temperature for 10 min., then centrifuged at 1000 x g for 10 min., resuspended in 20 μ l 0.1% DAPI ethanol. Following 15 min. incubation at room temperature, a 10 μ l aliquot was placed on a glass slide, and 400 cells per slide were scored for the incidence of apoptotic chromatin changes with a fluorescence microscope. A TdT-Mediated dUTP nick end labeling assay is used according to Gorczyca, W. et al., Cancer Res. 53, 1945 (1993) and Gavrieli, Y. et al., J. Cell Bio. 119, 493 (1992). An aliquot of 2×10^6 cells in 10 ml medium were respectively incubated with 2 μ l DMSO and 20 μ M noscapine (2 μ l 0.1 M DMSO solution) for 24 hr. Cells were pelleted and washed with ice-cold PBS twice, lymphocyte cells were fixed in 4% paraformaldehyde in PBS and air dried. The slides were rinsed with PBS and incubated with blocking solution (0.3% H_2O_2 in methanol) for 30 min. at room temperature. The slides were rinsed with PBS again and incubated in permeability solution (0.1% Triton x-100 in 0.1% sodium citrate) on ice for 2 min. Then the slides were washed twice with PBS, then 50 μ l nick end

labeling assay reaction mixture was added on samples and the slides were incubated in a humidified chamber for 60 min. at 37°C. After the slides were rinsed with PBS, 50 µl converter-POD solution was added on samples and incubated for 30 min. at 37°C. The slides were rinsed with PBS for 3 times, then 60 µl DAB substrate solution was added on the samples, and the slides were incubated at room temperature for 10 min. After the slides were rinsed with PBS for another 3 times, coverslips were mounted and analyzed with a light microscope.

Results show progressive DNA degradation with increasing time of noscapine treatment, as measured by gel electrophoresis of fragmented genomic DNA, or by staining of treated cells.

EXAMPLE 11

Inhibition of Tumor Growth by Noscapine

C57BL/6 mice were injected subcutaneously in the right flank with 2×10^6 E.G7-OVA cells. Three days later, mice were injected intraperitoneally, every day for three weeks, either with 200 µl saline (n=10), or with 3mg noscapine dissolved in 200 µl saline (n=10). Third group of mice (n=10) was fed 3 mg noscapine via intragastric (i.g.) intubation using a 1 ml syringe fitted with a 20 gauge stainless steel ball point needle. After three weeks, all mice were sacrificed by cervical dislocation. Tumors were removed and weighted. Tumor weights were individually plotted and comparisons between control and treatment groups were analyzed by the Student's t test. Statistical differences were considered significant if p values were less than 0.01. Results showed that mice treated with noscapine had significantly reduced tumor weight.

EXAMPLE 12

Noscapine Causes Apoptosis in Solid Lymphoid Tumors Induced in Mice

Microscopic examination of Haemotoxline and Eosine stained cells showed many cells in noscapine treated mice with apoptotic morphologies.

EXAMPLE 13

Noscapine Induces Conformational Change Upon Binding Tubulin and Promotes
Microtubule Assembly

Phosphocellulose purified bovine brain tubulin was employed throughout these biophysical experiments. Fluorescence titration for determining binding constants was performed according to Peyrot, V. et al., Biochemistry 31, 11125 (1992). In brief, at room temperature, 2 μ M tubulin in 100 mM PIPES, 2 mM EGTA, 1 mM $MgCl_2$ was excited at 278 nm, and the fluorescence emission spectra were recorded with bandwidths 2 nm. The fluorescence emission intensity of noscapine at this excitation wavelength was negligible and at the concentration of noscapine used it gave no appreciable inner filter effect. The concentration of noscapine was raised in increments of 0.5 μ M, until the decrease in the fluorescence intensity was saturated. The value of the dissociation constant and the number of sites were obtained from Scatchard plots using the equation, $r/[L]_{free} = n/K_d - r/K_d$, where r is the ratio of the concentration of bound ligand to the total protein concentration and n is the number of binding sites. Circular dichroism (CD) spectra measurements were performed in a spectrometer, in cells (0.1 cm path) at 25°C. Microtubule assembly was recorded on a spectrophotometer with thermocontroller. The cuvettes (0.4 cm path) containing 100 mM PIPES, 2 mM EGTA, 1 mM $MgCl_2$ and 1 mM GTP (G-PEM buffer), and 20 μ M noscapine/DMSO were kept at room temperature before addition of tubulin and shifting to 37°C. Tubulin and noscapine in G-PEM buffer did not show any detectable absorption at 350 nm. The assembly was monitored by measuring the changes in turbidity at 0.5 min. intervals. Noscapine was dissolved in DMSO at 0.8 mM and stocked at 4°C. The final concentration of DMSO was 2.5%.

Results show that noscapine affords fluorescence quenching of tubulin. Scatchard plot analysis showed an apparent dissociation constant (K_d) of $1.86 \pm 0.34 \times 10^{-6}$ M and a stoichiometry of 0.95 ± 0.02 noscapine molecule per complex of tubulin subunit. There is also saturation of the noscapine induced quenching in tubulin fluorescence intensity. Noscapine promotes tubulin assembly, as measured by increased absorbance at 350 nm of tubulin when treated with noscapine.

EXAMPLE 14

Initiation of Apoptosis by Noscapine and derivatives

Morphological changes in the nuclear chromatin of HL-60 cells undergoing apoptosis were detected by staining with 4', 6-diamidino-2-phenylindole (DAPI). In brief, 0.5×10^6 to 3×10^6 cells were fixed with 4% glutaraldehyde, 0.2% Triton x-100, in PBS and incubated at room temperature for 10 min., then centrifuged at $1000 \times g$ for 10 min., resuspended in $20 \mu\text{l}$ 0.1% DAPI ethanol. Following 15 min. incubation at room temperature, at $10 \mu\text{l}$ aliquot was placed on a glass slide, and 400 cells per slide were scored for the incidence of apoptotic chromatin changes with a fluorescence microscope.

Results show that noscapine, compound 3 and compound 4 initiate apoptosis.

| <u>Compound*</u> | <u>Apoptotic Cell Percentage</u> |
|-------------------------------------|----------------------------------|
| Noscapine, 20 μM in DMSO | 30, 17† |
| 3, 20 μM in DMSO | 37, 28 |
| 4, 20 μM in DMSO | 48, 32 |
| Noscapine, 50 μM in DMSO | 27 |
| 3, 50 μM in DMSO | 39 |
| 4, 50 μM in DMSO | 52 |

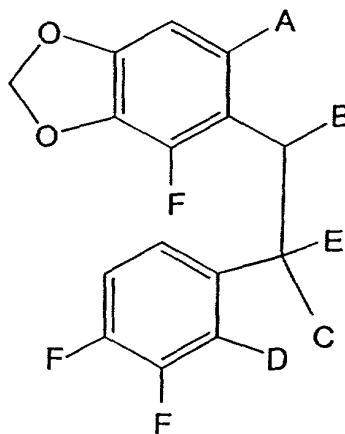
* All compounds were incubated with HL-60 cells at the indicated final concentrations for 24 hours.

† Two trials were conducted at 20 μM , the result for each trial is shown.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations, modifications or deletions as come within the scope of the following claims and its equivalents.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a compound of the formula



wherein: A is

- (i) $(\text{CH}_2)_n\text{-N}-\text{C}(\text{O})\text{O}-\text{C}_{1-6}\text{ alkyl}$; and W is $\text{C}_{1-6}\text{ alkyl}$; or
- (ii) $(\text{CH}_2)_2\text{-N}-$ and forms a six membered ring

with B, said ring containing one nitrogen;

Y is

- (a) $\text{C}_{1-6}\text{ alkyl}$, or H;
- (b) $\text{C}(\text{O})-\text{C}_{1-6}\text{ alkyl}$;
- (c) $\text{CH}_2\text{CH}(\text{OH})\text{-CH}_2\text{-Z}$, wherein Z is $\text{C}_{1-6}\text{ alkyl}$ or $\text{O}-\text{C}_{1-6}\text{ alkyl}$;

- (d) aryl; or
 (e) heterocycle;

B is a single bond, OH or halo;

C is -OH, -CH₂- or forms a 5-membered lactone or lactam ring with D; and

D is:

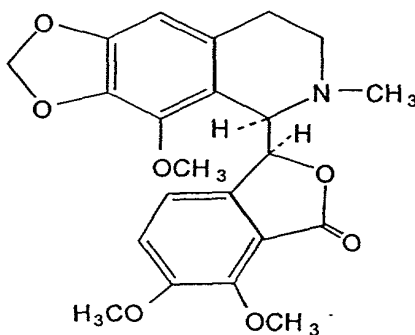
- (i) -OH, -CH₂-halo, -CH(0)-, -COOH, -C(O)-O-C₁₋₆ alkyl, -(CH₂)_n-,
 -CHOH-, wherein n is an integer and is 1,2, or 3; or

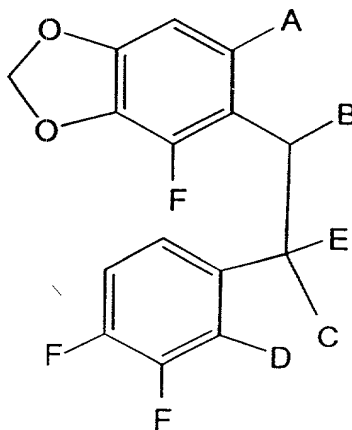
- (ii) forms a 5-membered lactone or lactam ring with C;

E is -H or -CH₃; and

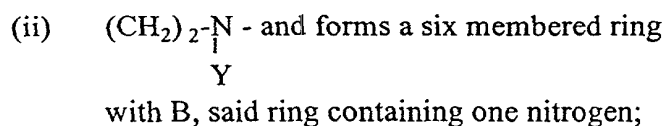
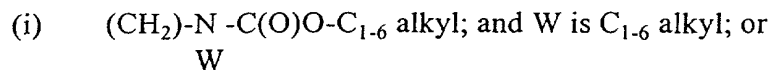
F is -OH,

or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, said composition useful in the treatment of neoplastic diseases, with the proviso that the formula excludes noscapine of the structure



[illegible]

wherein: A is



Y is

- (a) $\text{C}_{1-6}\text{ alkyl}$, or H;
- (b) $\text{C}(\text{O})-\text{C}_{1-6}\text{ alkyl}$;
- (c) $\text{CH}_2\text{CH}-\text{CH}_2-\text{Z}$, wherein Z is $\text{C}_{1-6}\text{ alkyl}$ or $\text{O}-\text{C}_{1-6}\text{ alkyl}$;
OH
- (d) aryl; or
- (e) heterocycle;

B is a single bond, OH or halo;

C is -OH, $-\text{CH}_2-$ or forms a 5-membered lactone or lactam ring with D; and

D is:

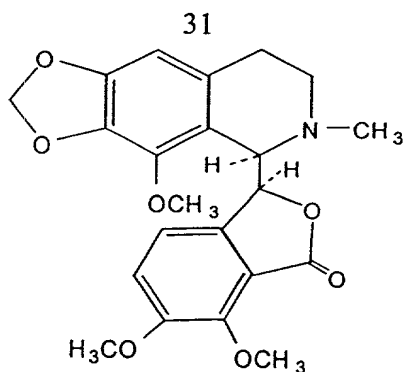
- (i) -OH, $-\text{CH}_2$ -halo, $-\text{CH}(\text{O})-$, $-\text{COOH}$, $-\text{C}(\text{O})-\text{O}-\text{C}_{1-6}$
alkyl, $-(\text{CH}_2)_n-$,
 $-\text{CHOH}-$, wherein n is an integer and is 1, 2, or 3; or

- (ii) forms a 5-membered lactone or lactam ring with C;

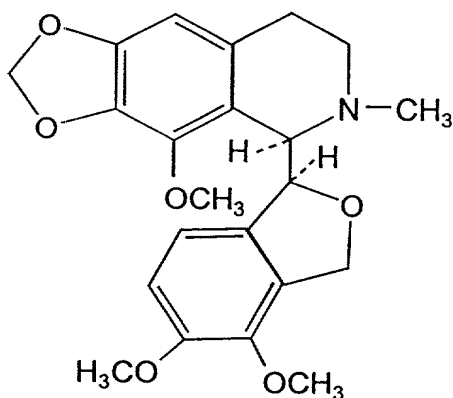
E is -H or $-\text{CH}_3$; and

F is -OH or $-\text{OCH}_3$,

or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, said composition useful in the treatment of neoplastic diseases, with the proviso that the formula excludes noscapine of the structure



4. A method for the treatment of neoplastic diseases, comprising administering to a mammal in need of such treatment an effective amount of a composition comprising a compound of the formula



or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, said composition useful in the treatment of neoplastic diseases.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
EMU144NAT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Noscapine Derivatives, Useful as Anticancer Agents

the specification of which (check only one item below):

- ☐ is attached hereto
- ☐ was filed as United States application
Serial No. _____
on _____
and was amended
on _____ (if applicable).
- ☒ was filed as PCT international application
Number PCT/US98/14979 on 20 July 1998,
and was amended under PCT Article 19
on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United State code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

| COUNTRY (of PCT indicate "PCT") | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED UNDER 35 USC 119 | |
|------------------------------------|--------------------|--------------------------------------|--------------------------------------|-----------------------------|
| | | | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| | | | <input type="checkbox"/> YES | <input type="checkbox"/> NO |

PTO 1391 (rev 10 83)

Page 1 of 3

US DEPARTMENT OF COMMERCE Patent and Trademark Office

(Combined Declaration For Patent Application and Power of Attorney --PTO 1391 [13-11]--page 1 of 2)

639632

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

| | | | | | |
|--|---------------------------|---|--|--|--|
| COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to PCT International Applications) | | | ATTORNEY DOCKET NUMBER EMU144NAT | | |
| <p>I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:</p> | | | | | |
| PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120: | | | | | |
| U.S. APPLICATIONS | | | STATUS (Check One) | | |
| U.S. APPLICATION NUMBER | U.S. FILING DATE | PATENTED | PENDING | ABANDONED | |
| 60/057,037 | 19 August 1997 (19.08.97) | | | X | |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO | PCT FILING DATE | U.S. SERIAL NUMBERS ASSIGNED (if any) | | | |
| | | | | | |
| | | | | | |
| <p>POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (<i>List name and registration number</i>) <u>Charles Y. Lackey 22,707; John M. Harrington 25,592; John S. Pratt 29,476; A. Jose Cortina 29,733; James L. Ewing, IV 30,630; Charles W. Calkins 31,814; George T. Marcou 33,014; Dean W. Russell 33,452; Richard T. Peterson 35,320; Charles T. Simmons 35,359; Nora M. Tocups 35,717; Bruce D. Gray 35,799; Theodore R. Harper 35,890; Kristin L. Johnson 44,807; Geoff L. Sutcliffe 36,348; Pat Winston Kennedy 36,970; Mitchell G. Stockwell 39,389; Michael J. Turton 40,852; Yoncha L. Kundupoglu 41,130; Benjamin D. Driscoll 41,571; Wilburn L. Chesser 41,668; Alana G. Kriegsman 41,747; J. Steven Gardner 41,772; James J. Bindseil 42,326; Richard H. Lilley 42,803; Edwina Thomas Washington 43,187; Camilla Camp Williams 43,992; Carl B. Massey P44,224; R. Whitney Winston P44,432; John William Ball, Jr. P44,433.</u></p> | | | | | |
| Send Correspondence to: <u>John S. Pratt, Esq.</u> <u>Kilpatrick Stockton LLP</u> <u>1100 Peachtree Street, Suite 2800</u> <u>Atlanta, GA 30309-4530</u> | | | Direct Telephone Calls to: (<i>name and telephone number</i>) <u>Bruce D. Gray</u> <u>(404) 815-6218</u> | | |
| 2 | FULL NAME OF INVENTOR | FAMILY NAME <u>Joshi</u> | FIRST GIVEN NAME <u>Harish</u> | SECOND GIVEN NAME <u>C.</u> | |
| 0 | RESIDENCE & CITIZENSHIP | CITY <u>Decatur</u> | STATE OR FOREIGN COUNTRY <u>Georgia</u> | COUNTRY OF CITIZENSHIP <u>US</u> | |
| 1 | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>1433 Oak Grove Drive</u> | CITY <u>Decatur</u> | STATE & ZIP CODE/COUNTRY <u>Georgia 30333 USA</u> | |
| 2 | FULL NAME OF INVENTOR | FAMILY NAME <u>Keqiang</u> | FIRST GIVEN NAME <u>Ye</u> | SECOND GIVEN NAME | |
| 0 | RESIDENCE & CITIZENSHIP | CITY <u>Atlanta</u> | STATE OR FOREIGN COUNTRY <u>Georgia</u> | COUNTRY OF CITIZENSHIP <u>China</u> | |
| 2 | POST OFFICE ADDRESS | POST OFFICE ADDRESS <u>2628 Northeast Expressway, Apt. N12</u> | CITY <u>Atlanta</u> | STATE & ZIP CODE/COUNTRY <u>Georgia 30345 USA</u> | |
| <p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.</p> | | | | | |
| SIGNATURE OF INVENTOR 201 <u>Harish C. Joshi</u> | | | SIGNATURE OF INVENTOR 202 <u>Keqiang Ye</u> | | |
| DATE <u>8/28/2000</u> | | | DATE | | |

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| | | | |
|--|--|---|---|
| COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to PCT International Applications) | | ATTORNEY DOCUMENT NUMBER BMUI44NAT | |
| I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application: | | | |
| PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120: | | | |
| U.S. APPLICATIONS | | STATUS (Check One) | |
| U.S. APPLICATION NUMBER | U.S. FILING DATE | PATENTED | PENDING |
| 60/057,037 | 19 August 1997 (19.08.97) | | <input checked="" type="checkbox"/> |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | |
| PCT APPLICATION NO | PCT FILING DATE | U.S. SERIAL NUMBERS ASSIGNED (if any) | |
| | | | |
| POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number) Charles Y. Lackey 22,707; John M. Harrington 25,592; John S. Pratt 29,476; A. Jose Cortina 29,733; James L. Ewing, IV 30,630; Charles W. Calkins 31,814; George T. Marcou 33,014; Dean W. Russell 33,452; Richard T. Peterson 35,320; Charles T. Simmons 35,359; Nora M. Tocups 35,717; Bruce D. Gray 35,799; Theodore R. Harper 35,890; Kristin L. Johnson 44,807; Geoff L. Sutcliffe 36,348; Pat Winston Kennedy 36,970; Mitchell G. Stockwell 39,389; Michael J. Turton 40,852; Yonchia L. Kundupoglu 41,130; Benjamin D. Driscoll 41,571; Wilburn L. Chesser 41,668; Alana C. Kriegsmann 41,747; J. Steven Gardner 41,772; James J. Bladsoil 42,326; Richard H. Lilley 42,803; Edwin Thomas Washington 43,187; Camilla Camp Williams 43,992; Carl E. Massey P44,224; R. Whitney Winston P44,432; John William Ball, Jr. P44,433 | | | |
| Send Correspondence to: John S. Pratt, Esq. Kilpatrick Stockton LLP 1100 Peachtree Street, Suite 2800 Atlanta, GA 30309-4530 | | Direct Telephone Calls to: (area code) Bruce D. Gray (404) 815-6218 | |
| 2 | FULL NAME OF INVENTOR FAMILY NAME Joshi | FIRST GIVEN NAME Harish | SECOND GIVEN NAME C. |
| 0 | RESIDENCE & CITIZENSHIP CITY Decatur | STATE OR FOREIGN COUNTRY Georgia | COUNTRY OF CITIZENSHIP US |
| 1 | POST OFFICE ADDRESS 1433 Oak Grove Drive | CITY Decatur | STATE & ZIP CODE/COUNTRY Georgia 30333 USA |
| 2 | FULL NAME OF INVENTOR FAMILY NAME Keqiang | FIRST GIVEN NAME Yc | SECOND GIVEN NAME Md |
| 0 | RESIDENCE & CITIZENSHIP CITY Atlanta | STATE OR FOREIGN COUNTRY Georgia | COUNTRY OF CITIZENSHIP China |
| 2 | POST OFFICE ADDRESS 262B Northeast Expressway, Apt. N12 | CITY Atlanta | STATE & ZIP CODE/COUNTRY Georgia 30345 USA |
| I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon. | | | |
| SIGNATURE OF INVENTOR 201 Harish C. Joshi | | SIGNATURE OF INVENTOR 202 Keqiang Ye | |
| DATE 06/20/00 | | DATE 06/20/00 | |

FIG 1191 (Rev 10-85)
Office

Page 2 of 3

US DEPARTMENT OF COMMERCE Patent and Trademark

6314 Greenspring Ave.
#401. (K.Y.E)

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COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

| | | | | | |
|---|--|--|---|--|---------|
| COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to PCT International Applications) | | | | ATTORNEY DOCKET NUMBER EMU144NAT | |
| I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application: | | | | | |
| PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120: | | | | | |
| U.S. APPLICATIONS | | | STATUS (Check One) | | |
| U.S. APPLICATION NUMBER 60/057,037 | U.S. FILING DATE 19 August 1997 (19.08.97) | PATENTED | PENDING | ABANDONED <div style="text-align: right;">X</div> | |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO | PCT FILING DATE | U.S. SERIAL NUMBERS ASSIGNED (if any) | | PATENTED | PENDING |
| | | | | | |
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| Send Correspondence to: John S. Pratt, Esq. Kilpatrick Stockton LLP 1100 Peachtree Street, Suite 2800 Atlanta, GA 30309-4530 | | | | Direct Telephone Calls to: (<i>name and telephone number</i>) Bruce D. Gray (404) 815-6218 | |
| 2 | FULL NAME OF INVENTOR JUDITH KAPP | FAMILY NAME Kapp | FIRST GIVEN NAME Judith | SECOND GIVEN NAME | |
| 0 | RESIDENCE & CITIZENSHIP Atlanta | CITY Atlanta | STATE OR FOREIGN COUNTRY Georgia | COUNTRY OF CITIZENSHIP US | |
| 1 | POST OFFICE ADDRESS 411 Chelsea Circle | POST OFFICE ADDRESS 411 Chelsea Circle | CITY Atlanta | STATE & ZIP CODE/COUNTRY Georgia 30307 USA | |
| 2 | FULL NAME OF INVENTOR LIU | FAMILY NAME Liu | FIRST GIVEN NAME Fuqiang | SECOND GIVEN NAME | |
| 0 | RESIDENCE & CITIZENSHIP Piscataway | CITY Piscataway | STATE OR FOREIGN COUNTRY New Jersey | COUNTRY OF CITIZENSHIP | |
| 2 | POST OFFICE ADDRESS 23 Marvin Lane | POST OFFICE ADDRESS 23 Marvin Lane | CITY Piscataway | STATE & ZIP CODE/COUNTRY New Jersey 08854 USA | |
| I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon. | | | | | |
| SIGNATURE OF INVENTOR 201 Judith Kapp <i>Judith Kapp</i> | | | SIGNATURE OF INVENTOR 202 Fuqiang Liu | | |
| DATE 3/14/00 | | | DATE | | |

| | | | | | |
|---|--|--|---|--|--|
| COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to PCT International Applications) | | | | ATTORNEY DOCKET NUMBER EMU144NAT | |
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| U.S. APPLICATIONS | | | STATUS (Check One) | | |
| U.S. APPLICATION NUMBER 60/057,037 | U.S. FILING DATE 19 August 1997 (19.08.97) | | PATENTED | PENDING | ABANDONED <div style="text-align: right;">X</div> |
| PCT APPLICATIONS DESIGNATING THE U.S. | | | | | |
| PCT APPLICATION NO | PCT FILING DATE | U.S. SERIAL NUMBERS ASSIGNED (if any) | | | |
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| 2 | FULL NAME OF INVENTOR JUDITH KAPP | FAMILY NAME Kapp | FIRST GIVEN NAME Judith | SECOND GIVEN NAME | |
| 0 | RESIDENCE & CITIZENSHIP | CITY Atlanta | STATE OR FOREIGN COUNTRY Georgia | COUNTRY OF CITIZENSHIP US | |
| 1 | POST OFFICE ADDRESS | POST OFFICE ADDRESS 411 Chelsea Circle | CITY Atlanta | STATE & ZIP CODE/COUNTRY Georgia 30307 USA | |
| 2 | FULL NAME OF INVENTOR FUQIANG LIU | FAMILY NAME Liu | FIRST GIVEN NAME Fuqiang | SECOND GIVEN NAME | |
| 0 | RESIDENCE & CITIZENSHIP | CITY Piscataway | STATE OR FOREIGN COUNTRY New Jersey | COUNTRY OF CITIZENSHIP China | |
| 2 | POST OFFICE ADDRESS | POST OFFICE ADDRESS 23 Marvin Lane | CITY Piscataway | STATE & ZIP CODE/COUNTRY New Jersey 08854 USA | |
| I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon. | | | | | |
| SIGNATURE OF INVENTOR 201 Judith Kapp | | | SIGNATURE OF INVENTOR 202 Fuqiang Liu | | |
| DATE | | | DATE March 30, 2000 | | |